Table 1. Strains and plasmids used.

Strain / plasmids	Description	Reference / source
S. coelicolor		
M145	Parental strain SCP1$^-$ SCP2$^-$	Hopwood *et al.* (1985)
T124	M145 (*accB*:pTR124), Th^R, Hyg^R	This work
T149	T124 containing pTR149 integrated in the *att* site of ϕC31, Th^R, Hyg^R, Am^R	This work
T149A	T149 with the wilde type *accB* copy of the chromosome replaced by the *accB*::*hyg* mutant allele, Hyg^R, Am^R	This work
E. coli		
DH5α	F$^-$ Δ*lacU169* (ϕ80*lacZ*ΔM15) *endA1 recA1 hsdR17 deoR supE44 thi-1* λ^- *gyrA96 relA1*	Hanahan (1983)
BL21λ(DE3)	F$^-$ *ompT* r_B^- m_B^- (DE3)	Studier & Moffatt (1986)
ET 12567	*supE44 hsdS20* ($r^-_Bm^-_B$) *ara-14 pro A2 lacY galK2 rpsL20 xyl-5 mtl-1 dam*$^-$ *dcm*$^-$ *hsdM*$^-$ Cm^R	MacNeil *et al.* (1992)
RG7	DH5α carrying pCL1 and pBA11 plasmids	Rodriguez & Gramajo (1999)
Plasmids		
pBluescript SK(+)	Phagemid vector (Ap^R *lacZ'*)	Stratagene
pGEM-T Easy	For cloning PCR products	Promega
pIJ2925	pUC18 derivative (Ap^R *lacZ'*)	Janssen & Bibb (1993)
pSET151	For the conjugal transfer of DNA from *E. coli* to *Streptomyces* spp. (Ap^R Th^R *lacZ'*)	Bierman *et al.* (1992)
pET22b(+)	Phagemid vector (Ap^R *lacZ'*) for expression of recombinant proteins under control of strong T7 transcription and translation signals	Novagen
pUZ8002	RK2 derivative with defective *oriT* (Km^R)	Paget *et al.* (1999)
pIJ8600	For the conjugal transfer of DNA from E. coli to Streptomyces spp. and for expression of recombinant proteins under *tipA* promoter	Sun *et al.* (1999)
pBA11	Vector containing *E. coli birA* gene	Barker & Campbell (1981)
pCL1	pSK(+) with a *Eco*RI-*Kpn*I insert carrying *accA1*	Rodriguez & Gramajo (1999)
pMR08	pSK(+) with a *Sst*I insert carrying *accBE*	This work
pTR88	pET22b(+) with *accBE* under control of strong T7 transcription and translation signals	This work
pTR90	pET22b(+) with *accB* under control of strong T7 transcription and translation signals	This work
pTR107	pET22b(+) with *accE* under control of strong T7 transcription and translation signals	This work
pTR124	pSET151 with a *hyg* (Hyg^R) gene inserted in the *accB* coding region	This work

pTR141	pIJ8600 derivative carrying *oriT* RK2, *ori* pUC18, *attP* site, *int* ϕC31 and *aac(3)IV* (Am^R)	This work
pTR149	pTR141 with a *KpnI* insert carrying *accBE*	This work

Table 2. Heterologous expresion of acyl-CoA carboxylase components in cell-free extracts of *E. coli* and *in vitro* reconstitution of enzyme activity

Strain	Proteins	Cell-free extracts	
E. coli *	induced by IPTG	ACCase [mU (mg protein) $^{-1}$]$^+$	PCCase [mU (mg protein)$^{-1}$]$^+$
RG7	AccA1+BirA	ND	ND
RG8	AccB, AccE	ND	ND
RG9	AccB	ND	ND
RG10	AccE	ND	ND
RG7:RG8 &	AccA1+BirA:AccB, AccE	2.35±0.06	3.10±0.07
RG7:RG9 &	AccA1+BirA:AccB	0.32±0.05	0.50±0.05
RG7:RG9:RG10 &	AccA1+BirA:AccB:AccE	1.38±0.05	1.77±0.06

ND, Not detectable. The amount of ^{14}C fixed into acid-stable products was not significantly higher than background levels (10-30 c.p.m., equivalent to 0.02-0.06 mU).

* All the RG strains are derived from *E. coli* DH5α

+ Results are means of three determinations ± SE

++ pBA11 expresses BirA constitutively

& Mix of equal amount of proteins from cell-free extracts from each of the strains indicated

Table 3 ACCase and PCCase activities in M145, M86 and M94

Strain *S. coelicolor*	Induction with Thiostrepton	Activity	
		ACCase [mU (mg protein)$^{-1}$]*	PCCase [mU (mg protein)$^{-1}$]*
M145	-	1.12±0.03	2.2±0.03
M86	-	0.43±0.03	1.45±0.06
M86	+	0.33±0.03	0.95±0.06
M94	-	0.40±0.03	1.57±0.03
M94	+	4.61±0.03 (11.5)	5.41±0.03 (3.5)

* Results are means of three determinations ± SE.

Table 4 Production of actinorhodin and undecylprodigiosin in YEME medium by M145 and M94.

Time (h)	M145		M94		[M94 +1µg Th]*		[M94 + 5 µg Th]*	
	Act	Red	Act	Red	Act (M)	Red (M)	Act (M)	Red (M)
40	-	-	-	-	$3x10^{-7}$	$10x10^{-7}$	$11x10^{-7}$	$28x10^{-7}$
60	-	-	-	-	$9x10^{-7}$	$12x10^{-7}$	$26x10^{-7}$	$78x10^{-7}$

* Results are mean of two indepent determinations.

(-) Means no detection of the antibiotics.

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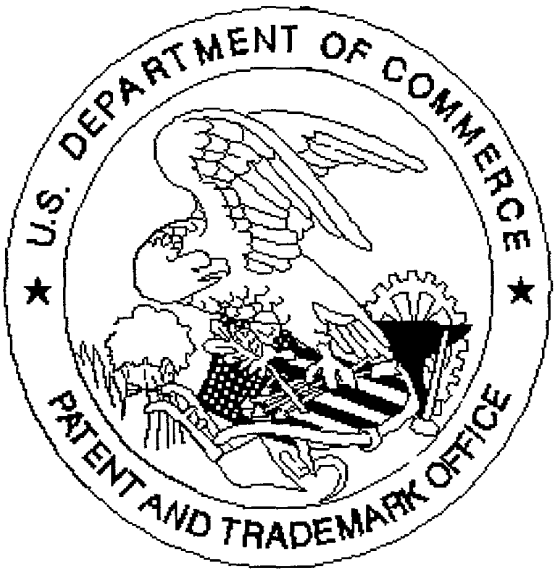

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